

University of Dundee

Novel biomarkers for risk stratification of Barrett's oesophagus associated neoplastic progression-epithelial HMGB1 expression and stromal lymphocytic phenotype

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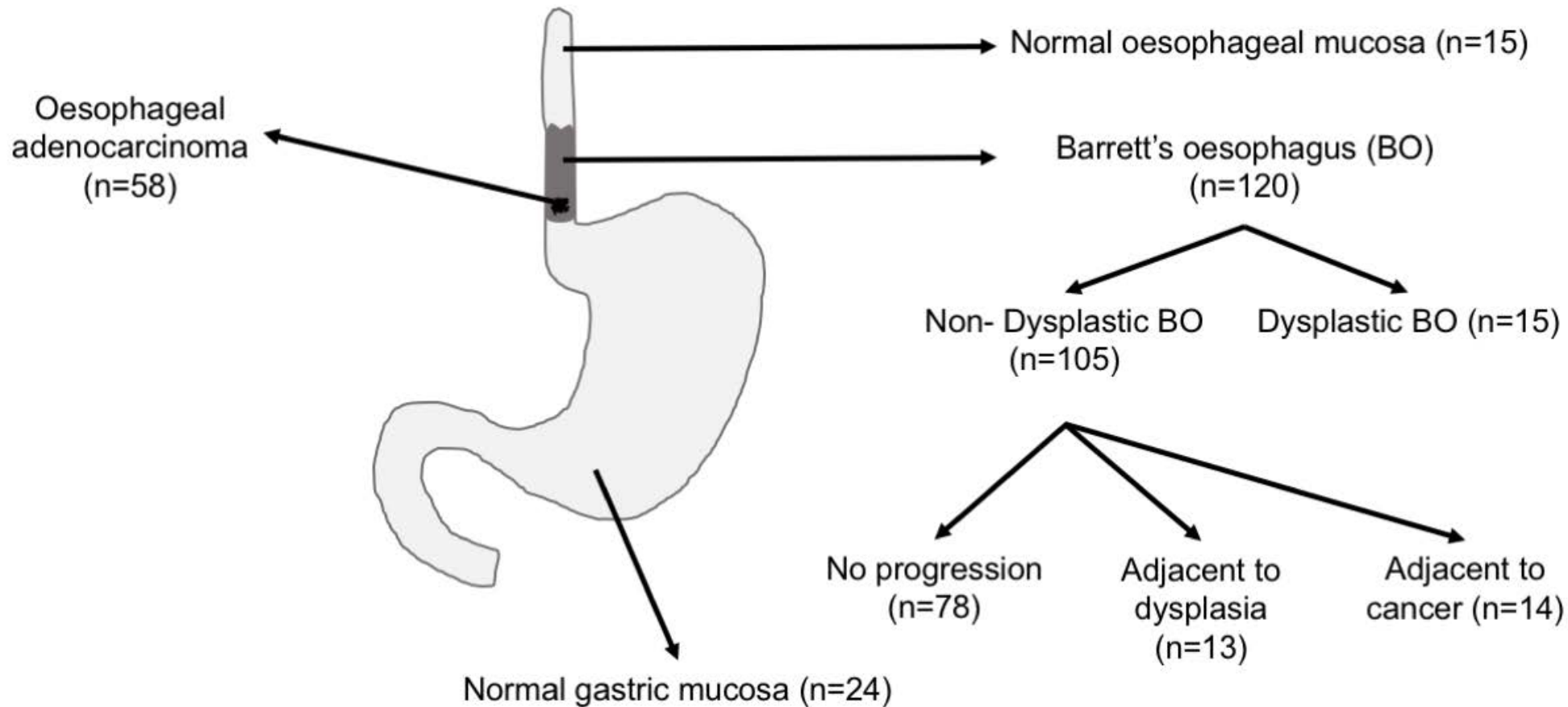
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Table 1: Association between epithelial target protein expression and histological diagnosis

Comparisons	HMGB1		p53		RUNX3	
	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm
Pre-malignant						
NO v NG	1	1	0.375	*	*	*
NO v ND BO	0.019	<0.001	1	0.584	*	*
NO v D BO	0.579	<0.001	<0.001	<0.001	*	*
NO v ND BO p-dys	0.568	<0.001	0.014	0.002	*	*
NO v ND BO p- OAC	1	0.295	1	*	*	*
NG v ND BO	0.018	<0.001	0.187	0.333	*	*
NG v D BO	0.622	<0.001	<0.001	<0.001	*	*
NG v ND BO p-dys	0.609	<0.001	<0.001	<0.001	*	*
NG v ND BO p-OAC	1	0.102	*	*	*	*
ND BO v D BO	0.137	0.177	<0.001	<0.001	*	*
ND BO v ND BO p-dys	0.211	0.509	0.001	0.001	*	*
ND BO v ND BO p-OAC	0.261	0.015	0.588	0.585	*	*
D BO v ND BO p-dys	1	0.598	0.05	0.462	*	*
D BO v ND BO p-OAC	1	0.004	<0.001	<0.001	*	*
ND BO p-dys v ND BO p-OAC	1	0.023	0.006	0.006	*	*
Oesophageal adenocarcinoma						
OAC v NO	0.116	0.002	0.002	1	1	*
OAC v NG	0.099	<0.001	<0.001	1	1	*
OAC v ND BO	0.232	0.001	<0.001	0.013	0.294	*
OAC v D BO	0.514	0.002	0.006	<0.001	1	*
OAC v ND BO p-dys	0.515	0.019	1	<0.001	1	*
OAC v ND BO p-OAC	0.682	0.49	0.001	1	1	*



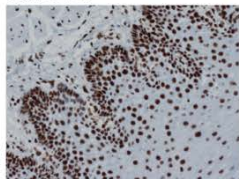
Normal Squamous
Epithelium

Non-Dysplastic
Metaplasia

Dysplastic
Metaplasia

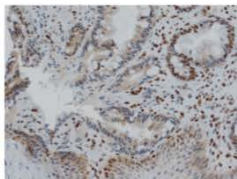
Oesophageal
Adenocarcinoma

HMGB1

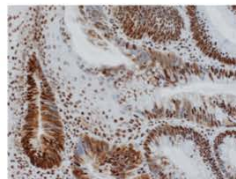


Nucleus

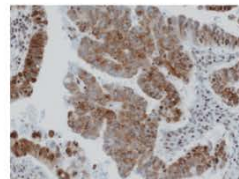
$p=0.019$



¹ $p=0.137$



² $p=0.514$



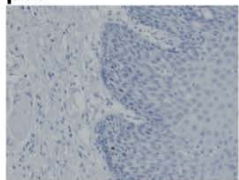
Cytoplasm

$p<0.001$

³ $p=0.177$

$p=0.022$

p53

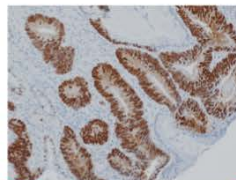


Nucleus

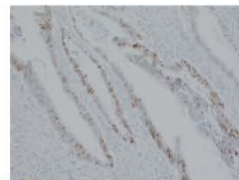
⁴ $p=1.000$



$p<0.001$



$p=0.006$



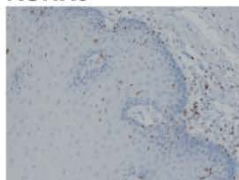
Cytoplasm

⁵ $p=0.584$

$p<0.001$

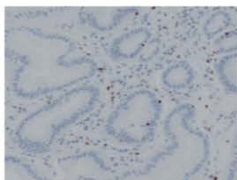
$p<0.001$

RUNX3

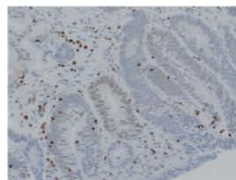


Nucleus

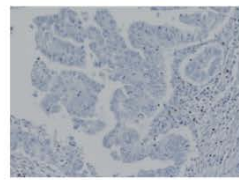
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⁶-



⁷-

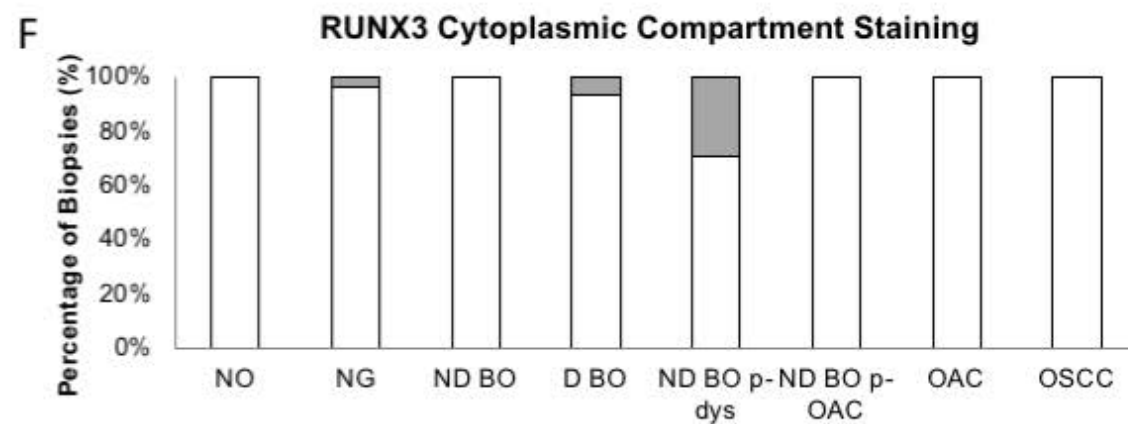
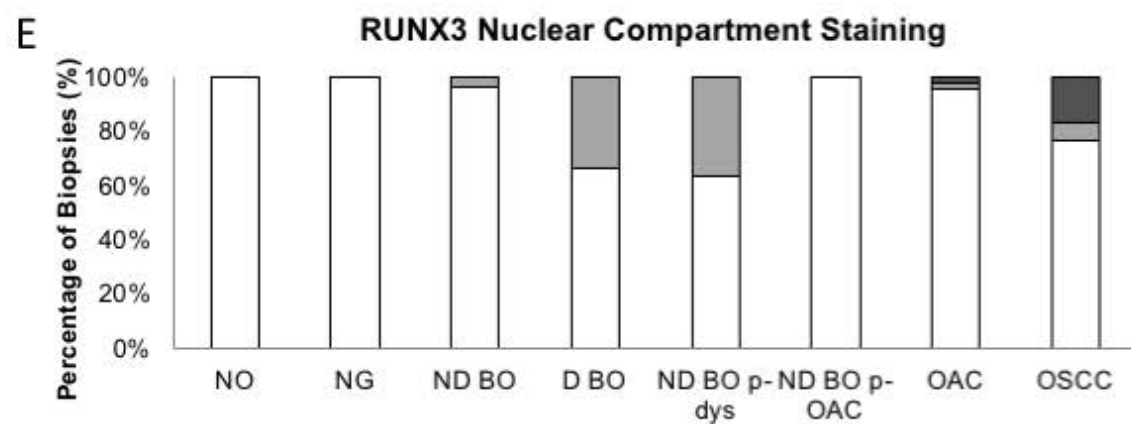
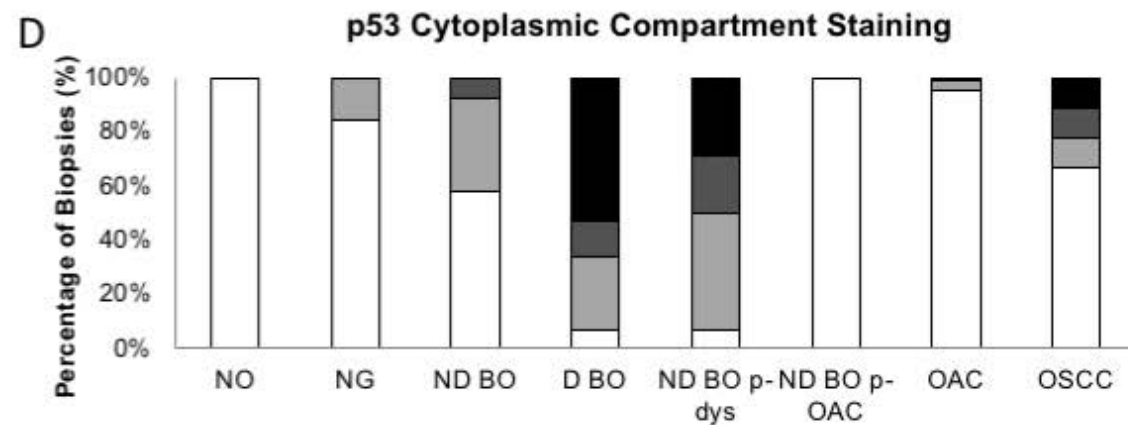
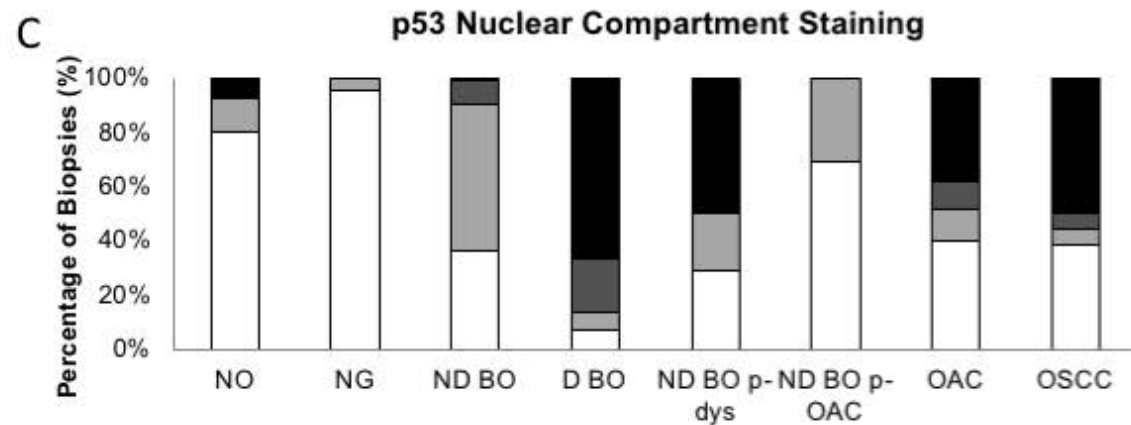
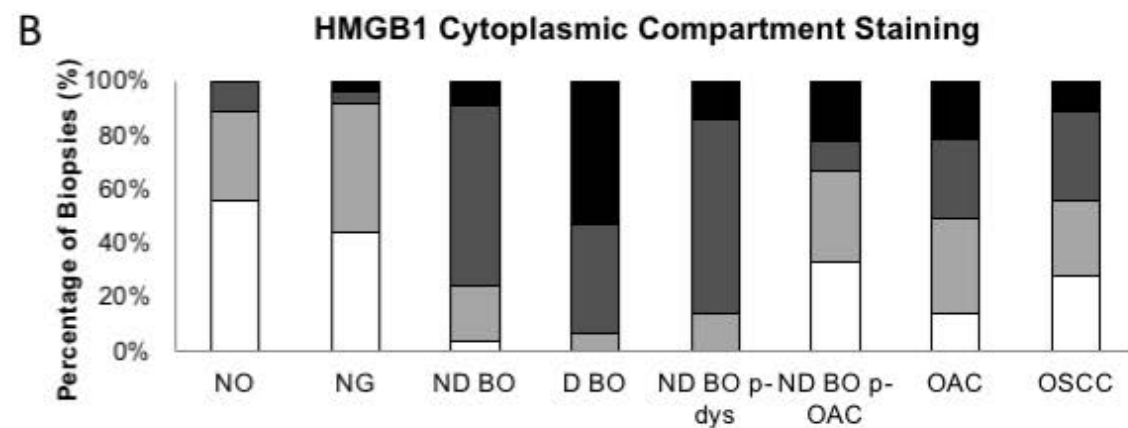
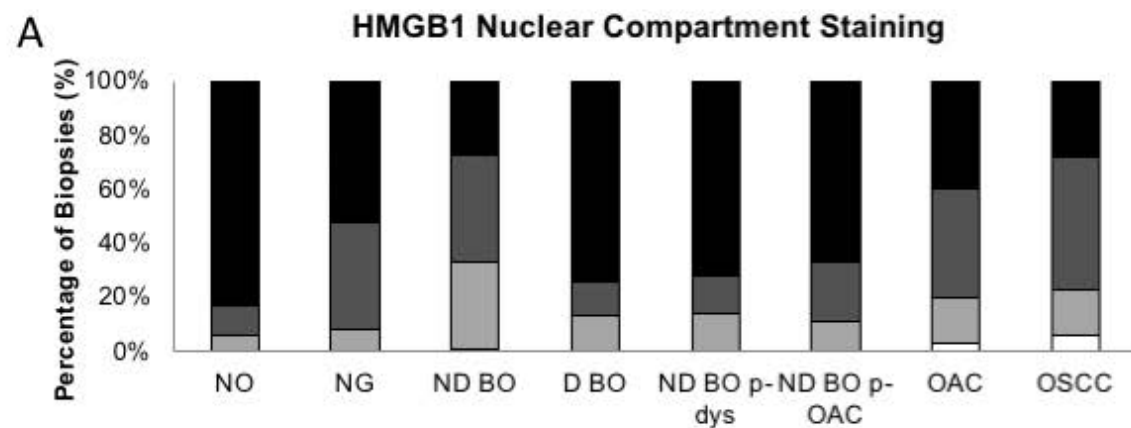


Cytoplasm

-

-

-



□ Absent □ Weak ■ Moderate ■ Strong

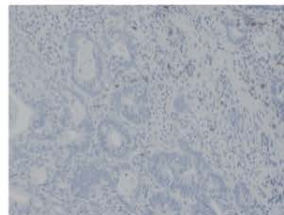
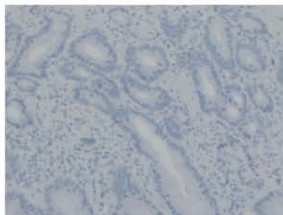
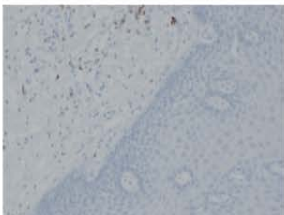
Normal Oesophagus

Non-Dysplastic BO

Dysplastic BO



CD20⁺
B-cells

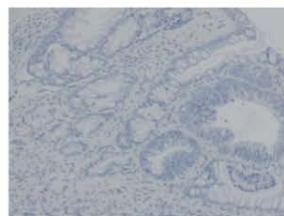
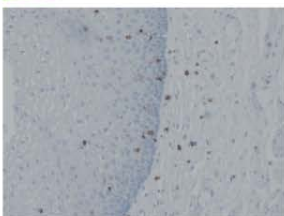


$p < 0.001$

$p = 0.003$



CD4⁺
T-cells

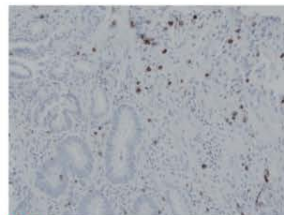
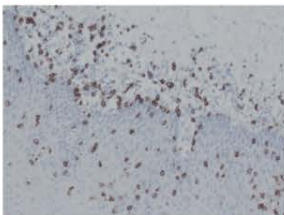


$p < 0.001$

$p = 0.430$



CD8⁺
T-cells

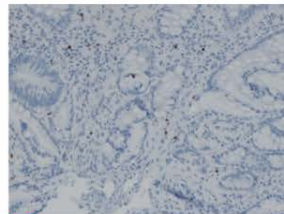
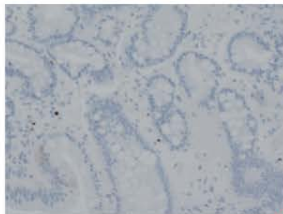


$p < 0.001$

$p = 0.012$



Foxp3⁺
Tregs



$p = 0.750$

$p < 0.001$



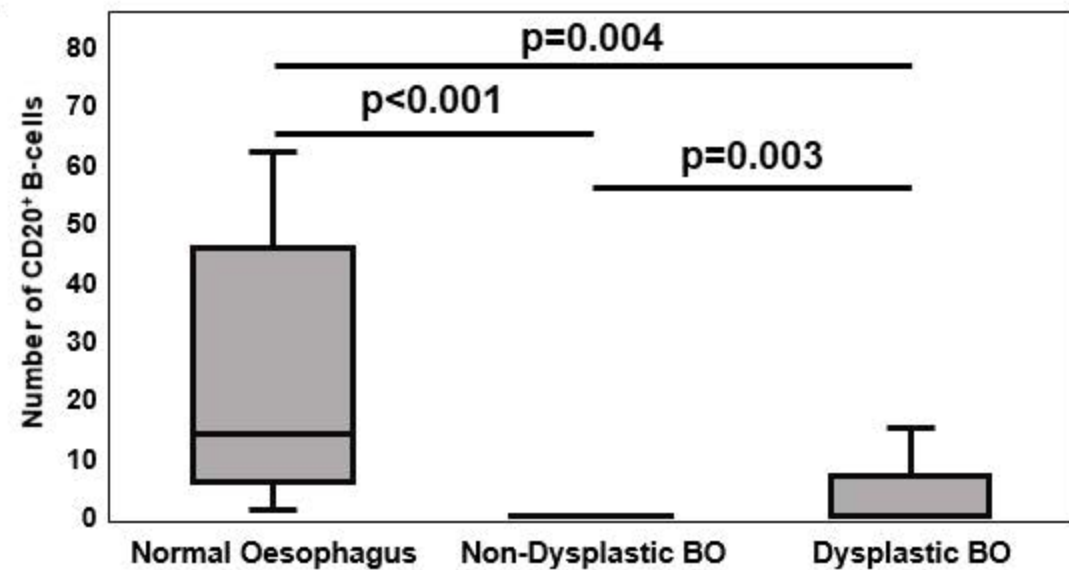
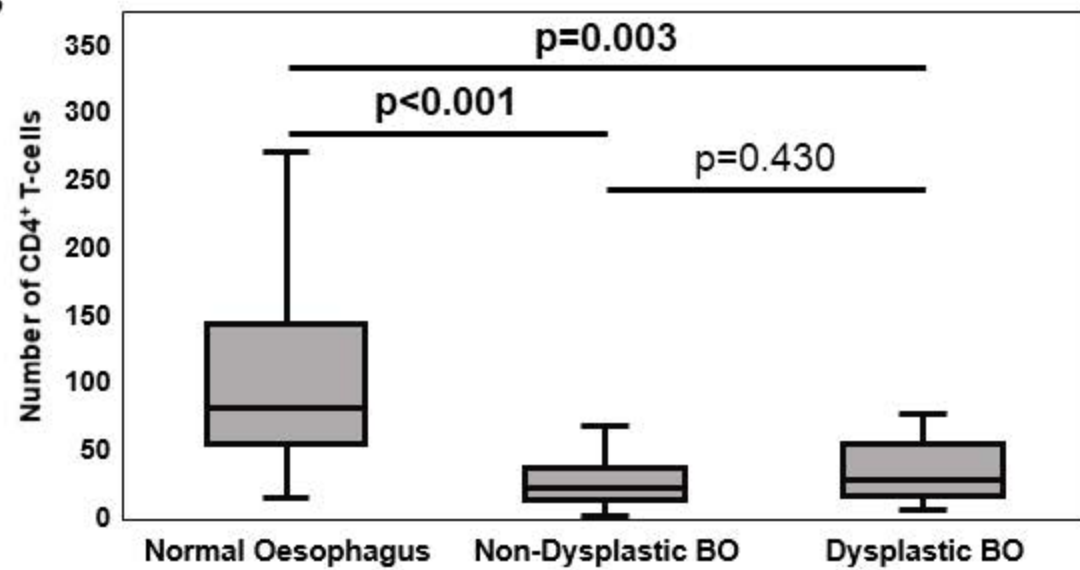
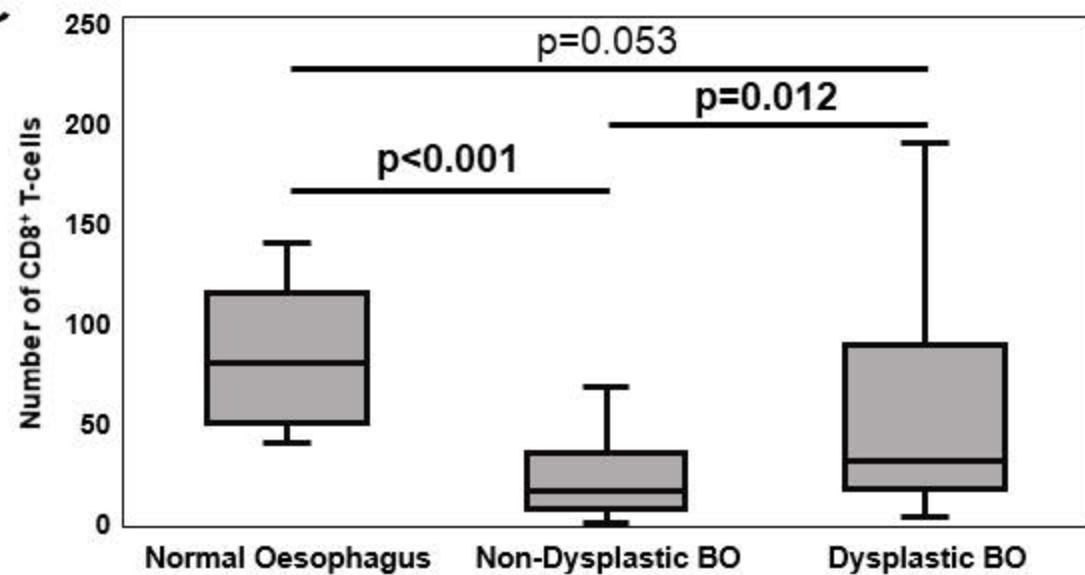
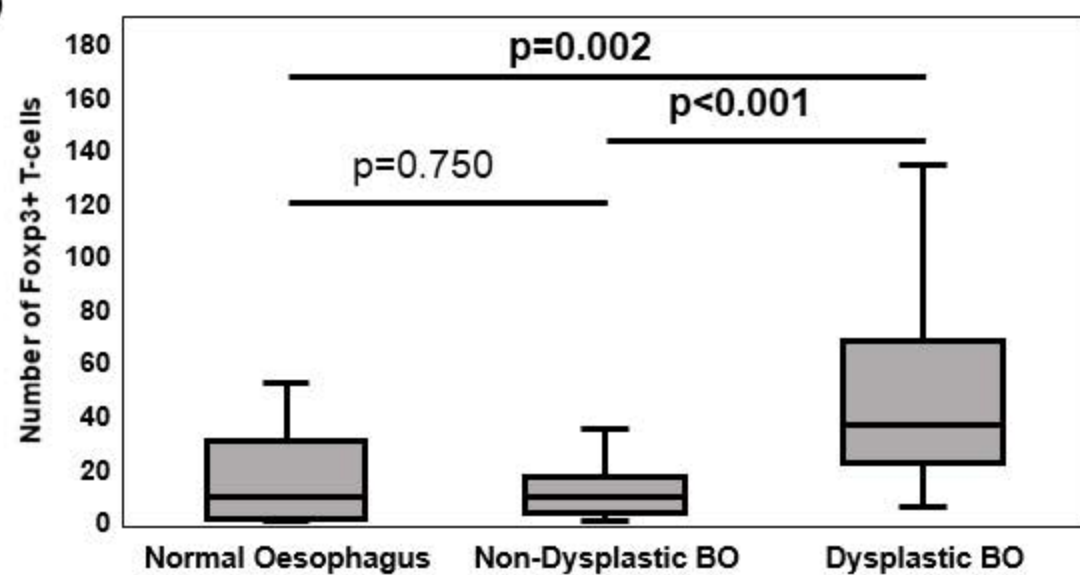
A**B****C****D**

Table S1: Patient characteristics and relationship between clinicopathological data and overall survival			
	Number of Patients (%)	2	p
Age at diagnosis*			
<65	17 (30%)	0.515	0.473
>65	40 (70%)		
Gender			
Male	12 (21%)	0.085	0.771
Female	46 (79%)		
pT Stage			
T1	12 (21%)	4.801	0.091
T2	13 (22%)		
T3	33 (57%)		
T4	0 (0%)		
N Stage			
N0	29 (50%)	5.279	0.071
N1	28 (48%)		
N2	1 (2%)		
N3	0 (0%)		
M Stage			
0	58 (100%)	-	-
1	0 (0%)		
Stage I-IV			
I (a/b)	13 (22%)	5.113	0.078
II (a/b)	19 (33%)		
III	26 (45%)		
IV	0 (0%)		
Chemotherapy Status**			
Received Chemotherapy	7 (13%)	2.003	0.157
Did not receive Chemotherapy	46 (87%)		
Mandard Regression Score***			
1	0 (0%)	4.428	0.219
2	2 (6%)		
3	6 (17%)		
4	16 (46%)		
5	11 (31%)		
30 Day Surgical Mortality*			
Alive >30 days	48 (84%)	90.767	<0.001
Alive <30 days	9 (16%)		
No data are available for *1, **5 and ***23 patients.			

Table 2: Characteristics of the antibodies used for immunohistochemistry

Antibody target	Antibody type	Antigen retrieval buffer	Dilution	Positive control	Supplier	Code	Isotype, clone	
HMGB1	rabbit monoclonal	citrate	1:400	colorectal cancer	abcam	ab79823	IgG, EPR3507	
p53	mouse monoclonal	EDTA	1:250	colorectal cancer	abcam	ab1101	IgG2a, DO-1	
RUNX3	mouse monoclonal	EDTA	1:500	colorectal cancer	abcam	ab40278	IgG1, R3-5G4	
CD20+ B-cells	mouse monoclonal	citrate	1:600	tonsil	Agilent Technologies	M 075529-2	IgG2a, L26	
CD4+ T-cells	mouse monoclonal	EDTA	1:500	tonsil		ab133616	IgG, EPR6855	
CD8+ T-cells	mouse monoclonal	EDTA	1:150	tonsil		abcam	ab17147	IgG1, 144B
Foxp3+ T-cells (Tregs)	mouse monoclonal	EDTA	1:200	tonsil		abcam	ab20034	IgG1, 236A/E7

Note: Citrate buffer at pH 6 and ethylenediaminetetraacetic acid (EDTA) at pH 7.8

Table S3: Association between HMGB1 and histological diagnosis; extended analysis

Comparrisons	Absent v weak v moderate v strong				Absent v weak, moderate and strong				Absent and weak v moderate and strong				Strong v absent, weak and moderate				Nuclear Relationship	Cytoplasmic Relationship
	Nucleus		Cytoplasm		Nucleus		Cytoplasm		Nucleus		Cytoplasm		Nucleus		Cytoplasm			
	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p		
Pre-malignant comparisons																		
NO v NG	4.811	0.078	2.320	0.652	*	*	-	0.543	-	1.000	-	1.000	-	0.052	-	1.000	-	-
NO v ND BO	18.257	<0.001	32.086	<0.001	-	1.000	-	<0.001	-	0.019	-	<0.001	-	<0.001	-	0.338	NO < ND BO	NO < ND BO
NO v D BO	0.885	0.303	24.723	<0.001	*	*	-	0.001	-	0.579	-	<0.001	-	0.674	-	<0.001	-	NO < D BO
NO v ND BO p-dys	1.052	0.270	19.497	<0.001	*	*	-	0.001	-	0.568	-	<0.001	-	0.669	-	0.183	-	NO < ND BO p-dys
NO v ND BO p- OAC	1.452	0.283	3.109	0.437	*	*	-	0.420	-	1.000	-	0.295	-	0.367	-	0.333	-	-
NG v ND BO	8.339	0.028	42.153	<0.001	-	1.000	-	<0.001	-	0.018	-	<0.001	-	0.028	-	0.675	NG < ND BO	NG < ND BO
NG v D BO	3.278	0.164	28.841	<0.001	*	*	-	0.003	-	0.622	-	<0.001	-	0.318	-	0.001	-	NG < D BO
NG v ND BO p-dys	2.930	0.268	24.883	<0.001	*	*	-	0.003	-	0.609	-	<0.001	-	0.317	-	0.289	-	NG < ND BO p-dys
NG v ND BO p-OAC	1.153	0.520	3.716	0.264	*	*	-	0.704	-	1.000	-	0.102	-	0.697	-	0.465	-	-
ND BO v D BO	10.745	0.008	13.196	0.002	-	1.000	-	1.000	-	0.137	-	0.177	-	0.002	-	<0.001	ND BO < D BO	ND < D BO
ND BO v ND BO p-dys	9.417	0.017	0.872	0.887	-	1.000	-	1.000	-	0.211	-	0.509	-	0.004	-	0.631	ND BO < ND BO p-dys	-
ND BO v ND BO p-OAC	5.625	0.164	10.397	0.008	-	1.000	-	0.015	-	0.261	-	0.015	-	0.024	-	1.000	ND BO < ND BO p-OAC	ND BO > ND BO p-OAC
D BO v ND BO p-dys	0.269	1.000	4.839	0.072	*	*	-	*	-	1.000	-	0.598	-	1.000	-	0.050	-	D BO > ND BO p-dys
D BO v ND BO p-OAC	0.625	0.822	9.591	0.015	*	*	-	0.042	-	1.000	-	0.004	-	1.000	-	0.080	-	D BO > ND BO p-OAC
ND BO p-dys v ND BO p-OAC	0.542	1.000	7.680	0.027	*	*	-	0.047	-	1.000	-	0.023	-	1.000	-	1.000	-	ND BO p-dys > ND BO p-OAC
Oesophageal adenocarcinoma																		
OAC v NO	17.973	<0.001	15.788	0.001	-	1.000	-	<0.001	-	0.116	-	0.002	-	<0.001	-	0.040	OAC < NO	OAC > NO
OAC v NG	5.630	0.111	17.701	<0.001	-	0.581	-	0.004	-	0.099	-	<0.001	-	0.030	-	0.044	OAC < NG	OAC > NG
OAC v ND BO	4.023	0.257	24.814	<0.001	-	0.392	-	0.037	-	0.232	-	0.001	-	1.000	-	0.039	-	OAC < ND BO
OAC v D BO	10.931	0.008	10.727	0.008	-	1.000	-	0.209	-	0.514	-	0.002	-	0.001	-	0.022	OAC < D BO	OAC < D BO
OAC v ND BO p-dys	9.543	0.017	8.844	0.020	-	1.000	-	0.209	-	0.515	-	0.019	-	0.002	-	0.731	OAC < ND BO p-dys	OAC < ND BO p-dys
OAC v ND BO p-OAC	4.782	0.142	2.245	0.563	-	1.000	-	0.152	-	0.682	-	0.490	-	0.024	-	0.682	OAC < ND BO p-OAC	-

Note. *no statistics are computed because one variable is a constant. - no Chi square value is provided as test was with a 2X2 contingency table

Table S4: Association between p53 and histological diagnosis; extended analysis

Comparrisons	Absent v weak v moderate v strong				Absent v weak, moderate and strong				Absent and weak v moderate and strong				Strong v absent, weak and moderate				Nuclear Relationship	Cytoplasmic Relationship
	Nucleus		Cytoplasm		Nucleus		Cytoplasm		Nucleus		Cytoplasm		Nucleus		Cytoplasm			
	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p	χ ²	p		
Pre-malignant comparisons																		
NO v NG	2.975	0.139	-	0.278	-	0.139	-	0.278	-	0.375	-	*	-	0.375	*	*	-	-
NO v ND BO	12.269	0.003	9.986	0.004	-	0.003	-	0.001	-	1.000	-	0.584	-	0.310	*	*	NO < ND BO	NO < ND BO
NO v D BO	20.483	<0.001	27.289	<0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-	0.002	-	0.002	NO < D BO	NO < D BO
NO v ND BO p-dys	8.561	0.010	25.755	<0.001	-	0.009	-	<0.001	-	0.014	-	0.002	-	0.014	-	0.042	NO < ND BO p-dys	NO < ND BO p-dys
NO v ND BO p- OAC	1.881	0.372	*	*	-	0.670	*	*	-	1.000	*	*	-	1.000	*	*	-	-
NG v ND BO	28.200	<0.001	5.391	0.057	-	<0.001	-	0.028	-	0.187	-	0.333	-	1.000	*	*	NG < ND BO	NG < ND BO
NG v D BO	36.226	<0.001	28.741	<0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	NG < D BO	NG < D BO
NG v ND BO p-dys	20.509	<0.001	24.677	<0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-	0.012	NG < ND BO p-dys	NG < ND BO p-dys
NG v ND BO p-OAC	-	0.038	-	0.278	-	0.038	-	0.278	*	*	*	*	*	*	*	*	NG < ND BO p-OAC	-
ND BO v D BO	41.842	<0.001	35.538	<0.001	-	0.031	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	ND BO < D BO	ND BO < D BO
ND BO v ND BO p-dys	23.019	<0.001	23.548	<0.001	-	0.001	-	0.001	-	0.001	-	0.001	-	<0.001	-	<0.001	ND BO < ND BO p-dys	ND BO < ND BO p-dys
ND BO v ND BO p-OAC	4.747	0.186	8.468	0.010	-	0.035	-	0.003	-	0.588	-	0.585	-	1.000	*	*	ND BO > ND BO p-OAC	ND BO > ND BO p-OAC
D BO v ND BO p-dys	5.679	0.140	2.164	0.723	-	0.169	-	1.000	-	0.050	-	0.462	-	0.462	-	0.264	D BO > ND BO p-dys	-
D BO v ND BO p-OAC	21.732	<0.001	24.793	<0.001	-	0.001	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-	0.002	D BO > ND BO p-OAC	D BO > ND BO p-OAC
ND BO p-dys v ND BO p-OAC	9.312	0.010	23.380	<0.001	-	0.057	-	<0.001	-	0.006	-	0.006	-	0.006	-	0.098	ND BO p-dys > ND BO p-OAC	ND BO p-dys > ND BO p-OAC
Oesophageal adenocarcinoma																		
OAC v NO	9.671	0.014	0.832	1.000	-	0.005	-	1.000		0.002	-	1.000	-	0.019	*	*	OAC > NO	-
OAC v NG	27.987	<0.001	4.877	0.102	-	<0.001	-	0.070		<0.001	-	1.000	-	<0.001	*	*	OAC > NG	-
OAC v ND BO	63.012	<0.001	39.655	<0.001	-	0.652	-	<0.001		<0.001	-	0.013	-	0.001	*	*	OAC > ND BO	OAC < ND BO
OAC v D BO	8.717	0.019	64.602	<0.001	-	0.010	-	<0.001		0.006	-	<0.001	-	0.051	-	<0.001	OAC < D BO	OAC < D BO
OAC v ND BO p-dys	2.834	0.416	57.393	<0.001	-	0.565	-	<0.001		1.000	-	<0.001	-	0.404	-	<0.001	-	OAC < ND BO p-dys
OAC v ND BO p-OAC	12.355	0.003	0.877	1.000	-	0.073	-	1.000		0.001	-	1.000	-	0.004	*	*	OAC > ND BO p-dys	-

Note. *no statistics are computed because one variable is a constant. - no Chi square value is provided as test was with a 2X2 contingency table

Table S5: Association between RUNX3 and histological diagnosis; extended analysis

Comparisons	Absent v weak v moderate v strong				Absent v weak, moderate and strong				Absent and weak v moderate and strong				Strong v absent, weak and moderate				Nuclear Relationship	Cytoplasmic Relationship
	Nucleus		Cytoplasm		Nucleus		Cytoplasm		Nucleus		Cytoplasm		Nucleus		Cytoplasm			
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p		
Pre-malignant comparisons																		
NO v NG	*	*	-	1.000	*	*	-	1.000	*	*	*	*	*	*	*	*	-	-
NO v ND BO	-	1.000	*	*	-	1.000	*	*	*	*	*	*	*	*	*	*	-	-
NO v D BO	-	0.013	-	0.455	-	0.013	-	0.455	*	*	*	*	*	*	*	*	NO < D BO	-
NO v ND BO p-dys	-	0.010	-	0.028	-	0.010	-	0.028	*	*	*	*	*	*	*	*	NO < ND BO p-dys	NO < ND BO p-dys
NO v ND BO p-OAC	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	-	-
NG v ND BO	-	1.000	-	0.276	-	1.000	-	0.276	*	*	*	*	*	*	*	*	-	-
NG v D BO	-	0.004	-	1.000	-	0.004	-	1.000	*	*	*	*	*	*	*	*	NG < D BO	-
NG v ND BO p-dys	-	0.003	-	0.039	-	0.003	-	0.039	*	*	*	*	*	*	*	*	NG < ND BO p-dys	NG < ND BO p-dys
NG v ND BO p-OAC	*	*	-	1.000	*	*	-	1.000	*	*	*	*	*	*	*	*	-	-
ND BO v D BO	-	0.001	-	0.174	-	0.001	-	0.174	*	*	*	*	*	*	*	*	ND BO < D BO	-
ND BO v ND BO p-dys	-	0.001	-	<0.001	-	0.001	-	<0.001	*	*	*	*	*	*	*	*	ND BO < ND BO p-dys	ND BO < ND BO p-dys
ND BO v ND BO p-OAC	-	1.000	*	*	-	1.000	*	*	*	*	*	*	*	*	*	*	-	-
D BO v ND BO p-dys	-	1.000	-	0.169	-	1.000	-	0.169	*	*	*	*	*	*	*	*	-	-
D BO v ND BO p-OAC	-	0.053	-	1.000	-	0.053	-	1.000	*	*	*	*	*	*	*	*	-	-
ND BO p-dys v ND BO p-OAC	-	0.046	-	0.105	-	0.046	-	0.105	*	*	*	*	*	*	*	*	ND BO p-dys > ND BO p-OAC	-
Oesophageal adenocarcinoma																		
OAC v NO	0.342	1.000	*	*	*	*	*	*	-	1.000	*	*	*	*	*	*	-	-
OAC v NG	0.407	1.000	-	1.000	-	0.586	-	0.185	-	1.000	*	*	*	*	*	*	-	-
OAC v ND BO	1.789	0.416	*	*	-	1.000	*	*	-	0.294	*	*	*	*	*	*	-	-
OAC v D BO	15.943	<0.001	-	0.112	-	0.002	-	0.112	-	1.000	*	*	*	*	*	*	OAC < D BO	-
OAC v ND BO p-dys	16.708	<0.001	-	<0.001	-	0.001	-	<0.001	-	1.000	*	*	*	*	*	*	OAC < ND BO p-dys	OAC < ND BO p-dys
OAC v ND BO p-OAC	0.586	1.000	*	*	-	1.000	*	*	-	1.000	*	*	*	*	*	*	-	-
Note: Two data points are computed because a significant association between nuclear and cytoplasmic liquidated as text with a 2x2 contingency table.																		

Note: *no statistics are computed because one variable is a constant. - no Chi square value is provided as test was with a 2X2 contingency table

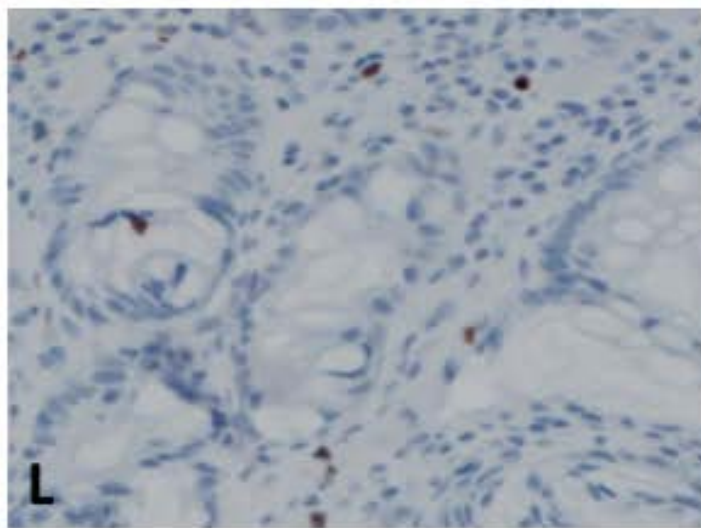
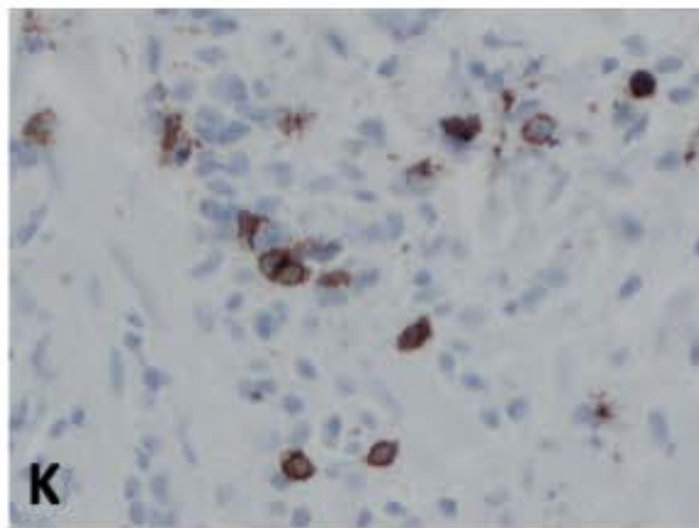
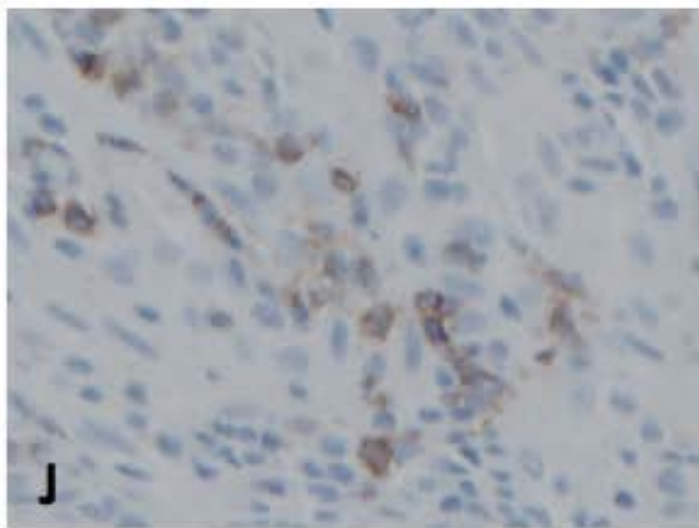
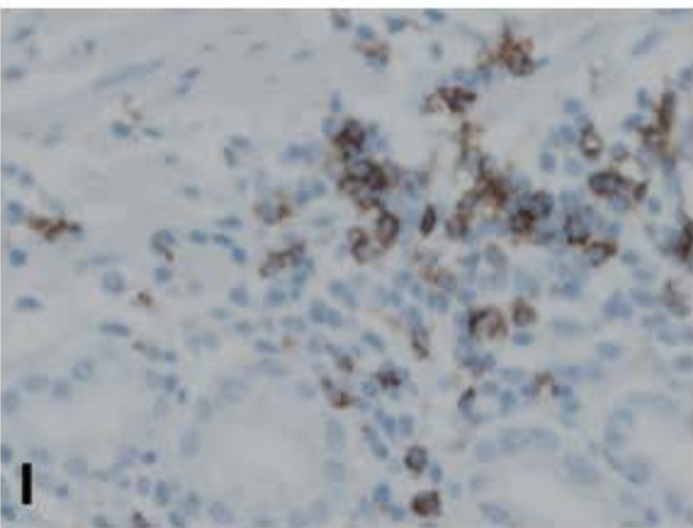
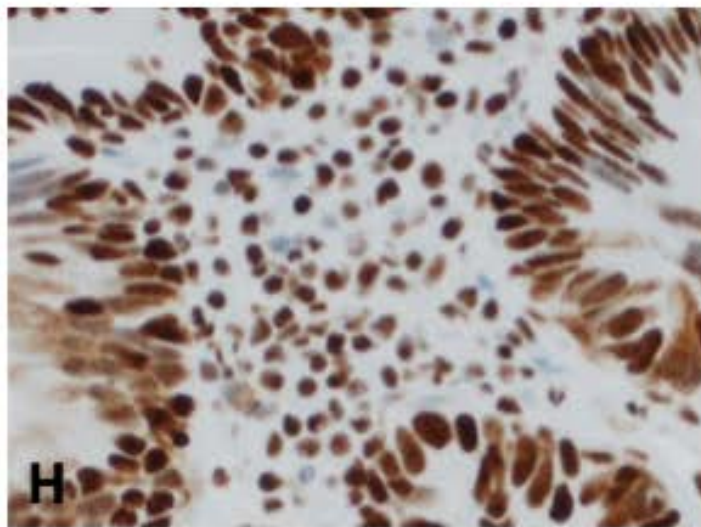
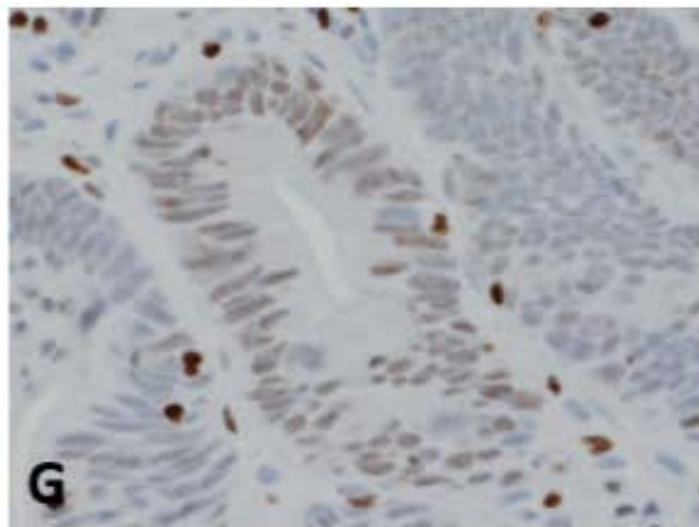
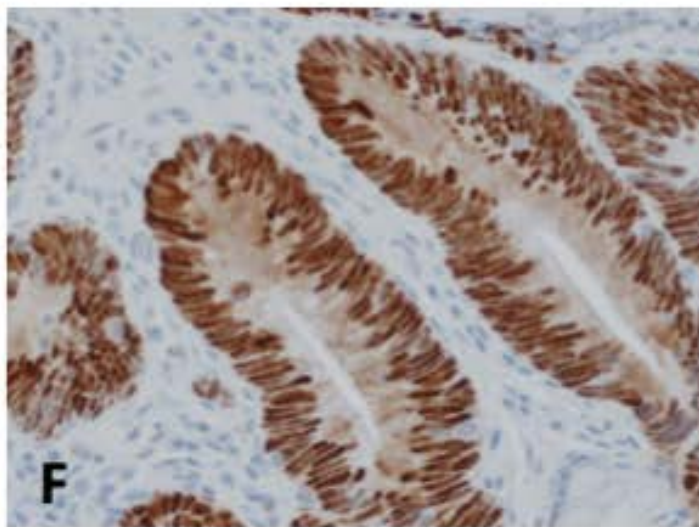
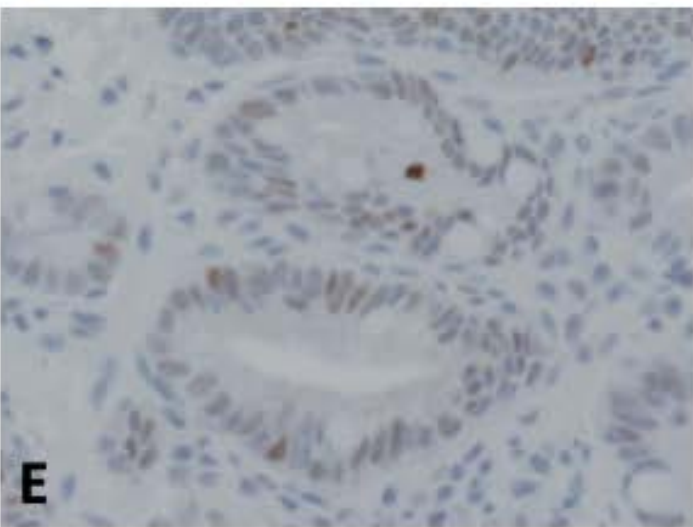
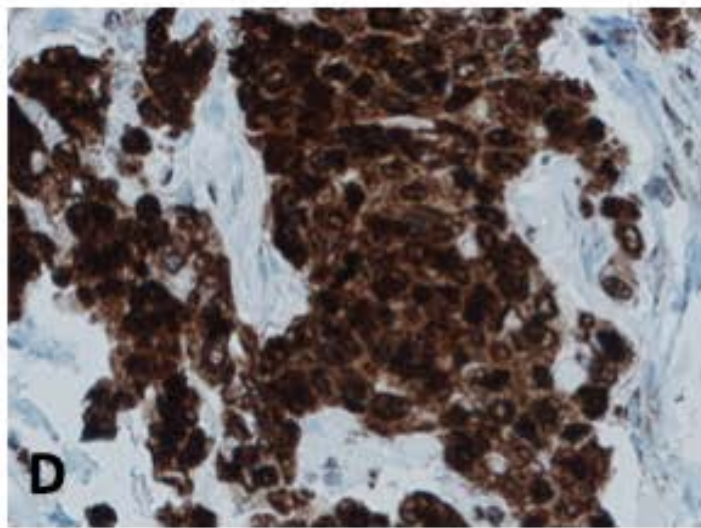
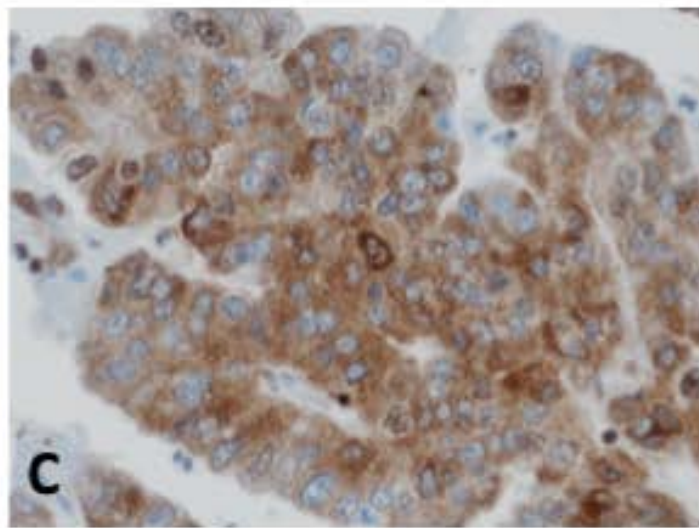
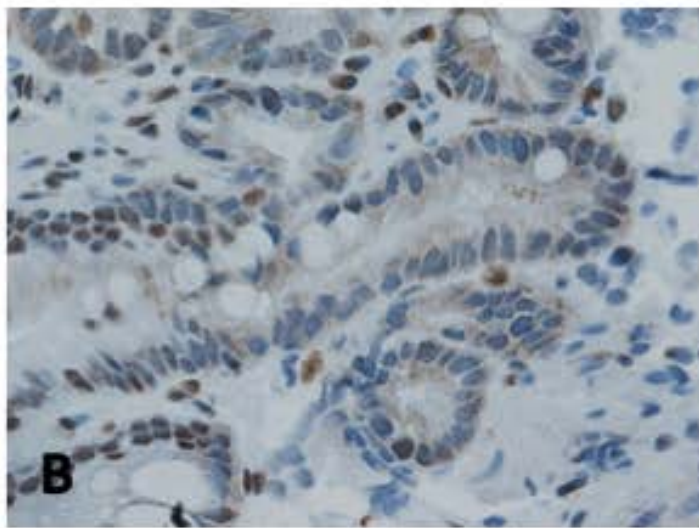
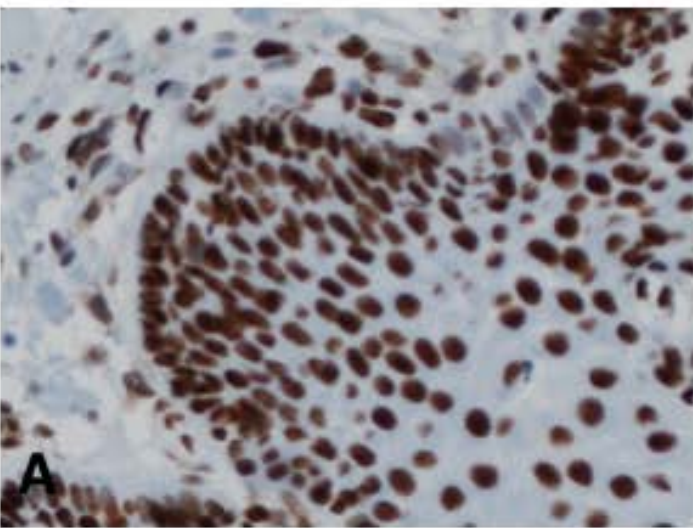
Table S6: Association between HMGB1, p53 and RUNX3 protein expression					
	0 v 1 v 2 v 3		0 v 1, 2, 3	0, 1 v 2 3	3 v 0, 1, 2
	χ^2	p	p	p	p
All Oesophageal Tissue (normal, Barrett's, dysplasia and cancer)					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	19.473	0.021	0.598	0.098	0.482
HMGB1 Nuclear vs. p53 Nuclear	7.655	0.569	0.412	0.432	0.767
HMGB1 Nuclear vs. RUNX3 Nuclear	10.391	0.109	1	0.34	-
p53 Nuclear vs. p53 Cytoplasmic	81.159	<0.001	<0.001	<0.001	<0.001
p53 Nuclear vs. RUNX3 Nuclear	22.871	0.001	0.002	0.024	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	85.269	<0.001	<0.001	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	25.13	0.003	0.004	0.013	0.018
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	3.308	0.347	1	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	44.32	<0.001	0.001	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	15.768	0.072	0.002	0.493	0.127
HMGB1 Cytoplasmic vs. RUNX3 Nuclear	8.336	0.205	0.085	0.688	-
Normal Oesophagus					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	2.88	0.578	-	1	-
HMGB1 Nuclear vs. p53 Nuclear	0.294	0.863	-	-	1
HMGB1 Nuclear vs. RUNX3 Nuclear	-	-	-	-	-
p53 Nuclear vs. p53 Cytoplasmic	-	-	-	-	-
p53 Nuclear vs. RUNX3 Nuclear	-	-	-	-	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	5.091	0.264	0.209	-	-
HMGB1 Cytoplasmic vs. RUNX3 Nuclear	-	-	-	-	-
All Barrett's oesophagus					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	25.055	0.003	1	0.006	0.008
HMGB1 Nuclear vs. p53 Nuclear	16.647	0.055	1	0.136	0.001
HMGB1 Nuclear vs. RUNX3 Nuclear	7.601	0.055	1	-	-
p53 Nuclear vs. p53 Cytoplasmic	87.008	<0.001	<0.001	<0.001	<0.001
p53 Nuclear vs. RUNX3 Nuclear	35.004	<0.001	0.053	-	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	43.196	<0.001	<0.001	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	16.218	0.062	1	0.778	0.004
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	1.134	0.72	1	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	21.534	<0.001	0.057	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	24.531	0.005	0.112	0.036	0.074
HMGB1 Cytoplasmic vs. RUNX3 Nuclear	0.583	1	1	-	-
All Non-dysplastic Barrett's oesophagus					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	22.723	0.007	1	0.001	0.379
HMGB1 Nuclear vs. p53 Nuclear	9.588	0.385	1	1	1
HMGB1 Nuclear vs. RUNX3 Nuclear	1.08	0.782	1	-	-
p53 Nuclear vs. p53 Cytoplasmic	25.891	<0.001	<0.001	0.096	-
p53 Nuclear vs. RUNX3 Nuclear	6.653	0.084	1	-	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	8.159	0.227	0.166	0.134	-
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	12.271	0.192	1	1	1

HMGB1 Cytoplasmic vs. RUNX3 Nuclear	3.855	0.278	1	-	-
Dysplastic Barrett's oesophagus					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	3.949	0.413	-	1	0.282
HMGB1 Nuclear vs. p53 Nuclear	9.909	0.129	-	1	0.56
HMGB1 Nuclear vs. RUNX3 Nuclear	5.182	0.075	-	-	-
p53 Nuclear vs. p53 Cytoplasmic	19.375	0.022	1	1	0.007
p53 Nuclear vs. RUNX3 Nuclear	3.75	0.29	1	-	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	2.143	0.143	0.333	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	8.984	0.174	-	1	0.619
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	15	<0.001	-	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	6.964	0.073	1	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	3.833	0.699	-	1	0.608
HMGB1 Cytoplasmic vs. RUNX3 Nuclear	4.313	0.116	-	-	-
Non-dysplastic Barrett's oesophagus in patients who have progressed to dysplasia					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	3.36	0.499	-	1	1
HMGB1 Nuclear vs. p53 Nuclear	6.8	0.147	-	0.462	0.559
HMGB1 Nuclear vs. RUNX3 Nuclear	3.111	0.211	-	-	-
p53 Nuclear vs. p53 Cytoplasmic	5.875	0.437	0.286	0.286	0.559
p53 Nuclear vs. RUNX3 Nuclear	3.63	0.163	0.221	-	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	10.08	0.001	0.005	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	4.9	0.557	-	0.462	0.505
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	2.24	0.326	-	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	5.833	0.12	1	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	12.367	0.015	-	-	0.462
HMGB1 Cytoplasmic vs. RUNX3 Nuclear	1.369	0.504	-	-	-
Non-dysplastic Barrett's oesophagus in patients who have progressed to adenocarcinoma					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	4	0.677	-	1	1
HMGB1 Nuclear vs. p53 Nuclear	0.875	0.646	-	-	-
HMGB1 Nuclear vs. RUNX3 Nuclear	-	-	-	-	-
p53 Nuclear vs. p53 Cytoplasmic	-	-	-	-	-
p53 Nuclear vs. RUNX3 Nuclear	-	-	-	-	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	7	0.072	1	-	-
HMGB1 Cytoplasmic vs. RUNX3 Nuclear	-	-	-	-	-
Oesophageal Adenocarcinoma					
HMGB1 Nuclear vs. HMGB1 Cytoplasmic	20.312	0.016	1	0.815	0.787
HMGB1 Nuclear vs. p53 Nuclear	10.197	0.335	0.293	0.816	0.491
HMGB1 Nuclear vs. RUNX3 Nuclear	3.289	0.772	1	1	-
p53 Nuclear vs. p53 Cytoplasmic	47.82	<0.001	0.08	1	-
p53 Nuclear vs. RUNX3 Nuclear	16.142	0.013	0.647	0.612	-
RUNX3 Nuclear vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Cytoplasmic	4.717	0.581	0.589	1	-
HMGB1 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
p53 Cytoplasmic vs. RUNX3 Cytoplasmic	-	-	-	-	-
HMGB1 Cytoplasmic vs. p53 Nuclear	5.664	0.773	0.37	0.242	1
HMGB1 Cytoplasmic vs. RUNX3 Nuclear	12.936	0.044	1	1	-
Note: - no Chi square value is provided as test was with a 2X2 contingency table					

Table S7: CD20+, CD4+, CD8+ and FOXP3+ lymphocytes in pre-malignant oesophageal neoplastic progression				
Histology	Number of samples	Median number of positive cells (SD)	25th percentile	75th percentile
CD20⁺ B-cells				
NO	10	14 (23.300)	5.75	45.75
NG	15	45 (38.221)	34	71
ND BO	70	0 (1.326)	0	0
D BO	13	0 (5.294)	0	7
ND BO p-dys	14	0 (4.480)	0	3.25
ND BO p-OAC	14	2 (15.745)	0	15.5
CD4⁺ T-cells				
NO	18	80 (66.261)	53.5	141.75
NG	18	57 (46.390)	26.75	92.5
ND BO	56	21 (21.186)	11	35.25
D BO	15	26 (39.071)	14	53
ND BO p-dys	12	20.50 (34.630)	12.25	68.25
ND BO p-OAC	11	35 (27.359)	31	64
CD8⁺ T-cells				
NO	13	80 (55.073)	49.5	114.5
NG	33	89 (53.242)	65.5	102.5
ND BO	61	16 (17.787)	7	34.5
D BO	15	31 (66.966)	17	89
ND BO p-dys	14	33.50 (41.589)	7.5	61.5
ND BO p-OAC	22	33 (27.399)	11.75	66.25
Foxp3⁺ T-cells (Tregs)				
NO	19	9 (16.305)	1	30
NG	16	5.50 (11.615)	2	10
ND BO	57	9 (10.348)	3	17
D BO	15	36 (34.117)	22	68
ND BO p-dys	13	11 (26.878)	3	33
ND BO p-OAC	16	8 (16.564)	1	28.75

Table S8: Association between lymphocyte populations and histological cell types.			
Comparisons	Mann Whitney-U	p	Relationship
CD20⁺ B-cells			
NO v NG	41.5	0.063	-
NO v ND BO	15	<0.001	NO > ND BO
NO v D BO	19.5	0.004	NO > D BO
NO v ND BO p-dys	14.5	0.001	NO > ND BO p-dys
NO v ND BO p-OAC	32	0.025	NO > ND BO p-OAC
NG v ND BO	48	<0.001	NG > ND BO
NG v D BO	15.5	<0.001	NG > D BO
NG v ND BO p-dys	14.5	<0.001	NG > ND BO p-dys
NG v ND BO p-OAC	28	<0.001	NG > ND BO p-OAC
ND BO v D BO	289	0.003	ND BO < D BO
ND BO v ND BO p-dys	374.5	0.038	ND BO < ND BO p-dys
ND BO v ND BO p-OAC	231	<0.001	ND BO < ND BO p-OAC
D BO v ND BO p-dys	78	0.478	-
D BO v ND BO p-OAC	76	0.445	-
ND BO p-dys v ND BO p-OAC	70.5	0.176	-
CD4⁺ T-cells			
NO v NG	110	0.1	-
NO v ND BO	11713	<0.001	NO > ND BO
NO v D BO	54	0.003	NO > D BO
NO v ND BO p-dys	42.5	0.006	NO > ND BO p-dys
NO v ND BO p-OAC	50.5	0.029	NO > ND BO p-OAC
NG v ND BO	219.5	<0.001	NG > ND BO
NG v D BO	82.5	0.058	-
NG v ND BO p-dys	60.5	0.044	NG > ND BO p-dys
NG v ND BO p-OAC	83	0.472	-
ND BO v D BO	364	0.43	-
ND BO v ND BO p-dys	303	0.595	-
ND BO v ND BO p-OAC	131	0.003	ND BO < ND BO p-OAC
D BO v ND BO p-dys	83.5	0.751	-
D BO v ND BO p-OAC	54	0.138	-
ND BO p-dys v ND BO p-OAC	35	0.056	-
CD8⁺ T-cells			
NO v NG	187	0.502	-
NO v ND BO	27.5	<0.001	NO > ND BO
NO v D BO	55.5	0.053	-
NO v ND BO p-dys	30	0.003	NO > ND BO p-dys
NO v ND BO p-OAC	42	0.001	NO > ND BO p-OAC
NG v ND BO	61.5	<0.001	NG > ND BO
NG v D BO	125	0.007	NG > D BO
NG v ND BO p-dys	67	<0.001	NG > ND BO p-dys
NG v ND BO p-OAC	83	<0.001	NG > ND BO p-OAC

ND BO v D BO	265	0.012	ND BO < D BO
ND BO v ND BO p-dys	311.5	0.116	-
ND BO v ND BO p-OAC	432	0.014	ND BO < ND BO p-OAC
D BO v ND BO p-dys	90	0.513	-
D BO v ND BO p-OAC	139	0.421	-
ND BO p-dys v ND BO p-OAC	147	0.82	-
Foxp3⁺ T-cells (Tregs)			
NO v NG	119.5	0.28	-
NO v ND BO	515	0.75	-
NO v D BO	54.5	0.002	NO < D BO
NO v ND BO p-dys	111.5	0.644	-
NO v ND BO p-OAC	151	0.974	-
NG v ND BO	356	0.181	-
NG v D BO	24	<0.001	NG < D BO
NG v ND BO p-dys	72	0.159	-
NG v ND BO p-OAC	108	0.45	-
ND BO v D BO	108.5	<0.001	ND BO < D BO
ND BO v ND BO p-dys	310.5	0.364	-
ND BO v ND BO p-OAC	452	0.957	-
D BO v ND BO p-dys	48	0.023	D BO > ND BO p-dys
D BO v ND BO p-OAC	44	0.003	D BO > ND BO p-OAC
ND BO p-dys v ND BO p-OAC	86	0.429	-



Novel biomarkers for risk stratification of Barrett's oesophagus associated neoplastic progression - epithelial HMGB1 expression and stromal lymphocytic phenotype

Figure legend Figure S1

Representative high power field photomicrographs representing (A) strong nuclear and absent cytoplasmic HMGB1 expression in normal oesophageal epithelium, (B) absent nuclear and weak cytoplasmic HMGB1 expression in non-dysplastic BO epithelium, (C) absent nuclear and moderate cytoplasmic HMGB1 expression in OAC, (D) strong nuclear and strong cytoplasmic HMGB1 expression in OAC, (E) weak nuclear p53 in non-dysplastic BO epithelium, (F) strong nuclear and moderate cytoplasmic p53 expression in dysplastic BO epithelium, (G) weak nuclear and absent cytoplasmic RUNX3 expression in dysplastic BO epithelium, (H) ubiquitous HMGB1 expression in stromal lymphocyte populations in dysplastic BO biopsy, (I) CD20 positive lymphocytes, (J) CD8 positive lymphocytes, (K) CD4 positive lymphocytes and (L) FOXP3 positive lymphocytes.